Open Access

J. Glob. Innov. Agric. Sci., 2024, 12(4):993-1001
ISSN (Online): 2788-4546; ISSN (Print):2788-4538
DOI: https://doi.org/10.22194/JGIAS/24.1503
http://www.jgiass.com

Society for Innovative Agiculture

Chitosan: Biocontrol of *Moniliophthora roreri* and a Biostimulant of *Theobroma cacao* L.

Juan Antonio Torres-Rodriguez[1], Juan José Reyes-Pérez[1], Gabriel José Medel- Rodriguez[2], Pablo Preciado Rangel[3*], Karla Nicole Factos Laiño[4] and Luis Guillermo Hernandez-Montiel[5,*]

[1]Universidad Técnica Estatal de Quevedo, Quevedo, Los Ríos, EC 120501, Ecuador; [2]Investigador independiente. Ángel Martínez Villareal 2721, Monterrey, Nuevo León, México. CP 64030; [3]Tecnológico Nacional de México/Campus Instituto; Tecnológico de Torreón. Carretera Torreón-San Pedro km 7.5, ejido Ana, Torreón, Coahuila, México. CP. 27170; [4]Instituto Nacional de Investigaciones Agropecuarias (INIAP), Mocache, Los Ríos, EC 170518, Ecuador; [5]Nanotechnology & Microbial Biocontrol Group, Centro de Investigaciones Biológicas del Noroeste, La Paz, Baja California Sur, 23096, México
*Corresponding author's e-mail: pablo.pr@torreon.tecnm.mx; lhernandez@cibnor.mx

Cocoa has considerable economic significance in Ecuador, playing an important role in agricultural sustainability and the national economy. The phytopathogen *Moniliophthora roreri* is responsible for frosty pod rot disease, which affects this crop. This study evaluated chitosan's role as a biostimulant and its effectiveness in controlling *Moniliophthora roreri* in cocoa cultivation. Five chitosan treatments (0.5, 1, 1.5, 2 g L^{-1}, and a control) were applied, and each treatment was replicated four times. The 2 g L^{-1} treatment significantly enhanced the number of pods (32.67) and almonds (66.5), along with increases in the fresh (1936.17 g) and dry (724.7 g) weights of the almonds compared to the control and lower chitosan concentrations. It also lowered the incidence and severity of *M. roreri* at 60 120 and 180 days. Using 2 g L^{-1} of chitosan also slowed down the disease (AUDPC) with a value of 1413.60 compared to the control treatment. These findings show that chitosan could be used as a long-term option in cocoa farming to boost yields and protection to *M. roreri.*

Keywords: Disease progression, incidence, efficiency, severity, yield.

INTRODUCTION

With Nacional and CCN-51 varieties, Ecuador ranks among the top global cocoa producers. These varieties have significant demand in international markets (Saravia-Matus *et al*., 2020; Salazar *et al*., 2023). For small- and medium-sized producers in Ecuador's rural areas, cocoa provides a critical income source essential for economic sustainability (Álava *et al*., 2021; Avadí, 2023). However, these producers currently face substantial challenges due to phytopathogens like *Moniliophthora roreri*, which adversely affect both quality and yield (Gómez-de la Cruz *et al*., 2023).

In light of these challenges, it is imperative to identify environmentally favorable alternatives that can enhance production (Goudsmit *et al*., 2023; Torres-Rodriguez *et al*., 2024a). In this context, chitosan can be an alternative due to its biodegradability (Mohan *et al*., 2022).

Chitosan can be used as a biocontrol agent as shown by more than one study. Through the interaction of glucosamine's amino groups (NH^{3+}) with the components of microorganisms' cell walls, such as phospholipids in fungi's cell membranes, chitosan inhibits the growth of phytopathogens (Ardean *et al*., 2021; Torres-Rodriguez *et al*., 2021). It also helps plants activate their defense systems by producing reactive oxygen species (ROS), phytoalexins, pathogenesis-related (PR) proteins, and defensive enzymes (Suwanchaikasem *et al*., 2023; Gong *et al*., 2024).

Chitosan has also been shown to increase plant growth and development. This bipolymer not only helps plants grow and develop faster, but it also increases output by improving things like the number of fruits, their size, and their weight (Gustavo González *et al*., 2015; Munaro *et al*., 2024). These positive effects of chitosan on plants are related to the ability of chitosan to increase the activity of enzymes related to the processes of photosynthesis (Sun *et al*., 2023). Chitosan application has also been shown to increase the uptake of nutrients essential for plant growth and development (Faluku *et al*., 2024). However, despite the research carried out on chitosan, few works evaluate its effect as a biocontrol agent and biostimulant in cocoa cultivation.

Rodriguez, J.A.T., J.J.R. Pérez, G.J.M. Rodriguez, P.P. Rangel, K.N.F. Laiño and L.G.H. Montiel. 2024. Chitosan: Biocontrol of *Moniliophthora roreri* and a Biostimulant of *Theobroma cacao L*. Journal of Global Innovations in Agricultural Sciences 12:993-1001.
[Received 16 Aug 2024; Accepted 2 Nov 2024; Published 17 Nov 2024]

MATERIALS AND METHODS

***Experimental area location*:** The research was carried out at the El Guineo farm, in the El Tigre district of the Buena Fe canton, with geographic coordinates 0°55'05.2" South latitude and 79°27'36.5" West longitude, at an altitude of 96 meters above mean sea level. This region has a humid tropical climate, average annual temperatures of 25.30 °C, an average annual rainfall of 1,640.30 mm and a relative humidity of 87%.

Genetic material: The highly productive clone CCN-51 was used. This clone has a high percentage of pods per kilogram of dry cocoa. It is an erect plant with a relatively low height that facilitates agricultural activities such as pruning and harvesting (Crespo and Crespo, 1997; Fuentes and Castelblanco, 2011).

***Treatment and experimental design*:** This study evaluated the effects of four chitosan concentrations as biostimulants and biocontrol agents against *M. roreri* in cocoa cultivation, along with a control treatment (no application). A randomized complete block design was used, comprising five treatments with four replications. The treatments included: T1: (0.5 g L^{-1}), T2: (1 g L^{-1}), T3: (1.5 g L^{-1}), T4: (2 g L^{-1}), and T5: (control treatment – water application).

***Application method*:** Chitosan was applied at the onset of flowering when more than 50% of cocoa plants exhibited flowers. Subsequently, applications were made every 30 days, resulting in six applications over a period of 180 days. The application method used a CP3 backpack sprayer with a capacity of 20 L, employing vertical movements of the rod while circling the plant canopy to ensure uniform distribution across the leaf area. The distance between the backpack rod and the plant canopy was approximately 1 m.

Evaluated variables

***Production and yield indicators*:** The number of pods (NP) was recorded for 20 plants per treatment by counting during the evaluation period. To determine the fresh weight of almonds (FWB) in g, 25 pods were randomly selected from the harvested pods of 20 plants per treatment, and all almonds were extracted and weighed using a digital scale (Santorius, 2 Kg±1g). To determine dry weight, almonds were dried in an oven (Memmert, 60 °C, 72 hours) and subsequently weighed until a constant weight was achieved, indicating complete removal of moisture from the samples. Yield was estimated in tons of dry cocoa almonds, calculated as 40% of the fresh weight of cocoa, by multiplying the fresh weight per plant by 0.40 (conversion factor for obtaining dry weight).

***Disease incidence (DI) by M. roreri in cocoa cultivation*:** To assess the incidence of disease (DI), the numbers of diseased and healthy pods were counted. DI was calculated as the proportion of infested pods exhibiting disease symptoms, such as spots and necrosis, relative to the total number of pods. Disease incidence was evaluated at 60, 120, and 180 days (Torres-Rodriguez *et al*., 2024b). The DI percentage was calculated using the following formula:

$DI = Di/Dh \times 100$

Where DI = Disease incidence (%); Di = Diseased pods, and Dh = Healthy pods

***Severity (SV) of M. roreri in cocoa pods*:** To evaluate the severity of the disease in the pods of each plant, the degree of infection was assessed using the arbitrary scale of 1–5 proposed by Villamil Carvajal (2015), with modifications (Table 1). The severity (SV) of the fruits was evaluated based on visible symptoms such as oily spots, necrosis, and sporulation. Disease severity was assessed at 60, 120, and 180 days.

Table 1. Scale of disease severity in cocoa fruit

Severity scale	Description
0	No apparent symptoms
1	Small and few oily spots
2	Well-defined and abundant oily spots along with more deformation or irregular ripening
3	Necrosis without sporulation
4	Necrosis with sporulation in an area of less than one-fourth of the necrotic surface
5	Necrosis with sporulation in an area greater than one-fourth of the necrotic surface in relation to the percentage of affected tissues on the pod exterior.

To determine the external severity of the infection, the following formula was used:

$$SE\ (\%) = [\sum (SS \times NS)/(N \times K)] \times 100$$

where SS = Disease scale score (0-5), NS = Number of pods at the score level, N = Total number of pods evaluated, and K = Maximum scale value.

***Treatment efficiency*:** The efficiency of chitosan application against the phytopathogen *M. roreri* in cocoa cultivation was assessed using the following formula proposed by Abbott (1925):

$$E = ((FIWoQ - FIWQ)/FIWoQ) \times 100$$

where E = Efficiency (%); FIWoQ = Disease severity in the control (without chitosan); FIWQ= Disease severity with chitosan application.

***Effect of chitosan on the area under the disease progress curve (AUDPC) of M. roreri in cocoa*:** To evaluate the effect of chitosan on the area under the disease progress curve (AUDPC), disease severity was recorded at various time points throughout the experiment, specifically on designated days after sowing (DAS). Severity was quantified using a symptom scale, and these data were subsequently used to calculate the AUDPC using the following formula (Zhao *et al*., 2012).

$$AUDPC = \sum_{i=1}^{n-1} [(DS_i + DS_{i+1})/2] \times (t_{i+1} - t_i)$$

where DS_i= The disease severity value at the specific time point t_i; DS_{i+1}= The disease severity value at the next time points i+1; t_i = Time in specific days after sowing (DAS) at point i; t_{i+1}= Time in specific days after sowing (DAS) at the next point i+1.

***Statistical analysis*:** Data normality was assessed using the Shapiro-Wilk test, whereas homogeneity of variance was verified using the Bartlett test. Data analysis was performed using one-way analysis of variance (ANOVA) using STATISTICA 10.0 software, and Tukey's multiple comparison tests for means were performed, considering a significance level of $p < 0.05$.

RESULTS

***Impact of chitosan on the number of cocoa pods*:** Analysis of variance (ANOVA) showed significant differences in the number of cocoa pods. Chitosan treatment outperformed the control treatment (no application) regarding the number of cocoa pods. The highest outcome was recorded using chitosan treatment 2 g L^{-1}, resulting in a mean of 32.67 pods, which exhibited significant differences ($p < 0.05$) compared to the other treatments. The lowest results were observed with the 1 and 0.5 g L^{-1} treatments; however, both concentrations were higher than the control. The control treatment yielded the lowest number of pods, indicating that chitosan application boosted the number of cocoa pods (Figure 1).

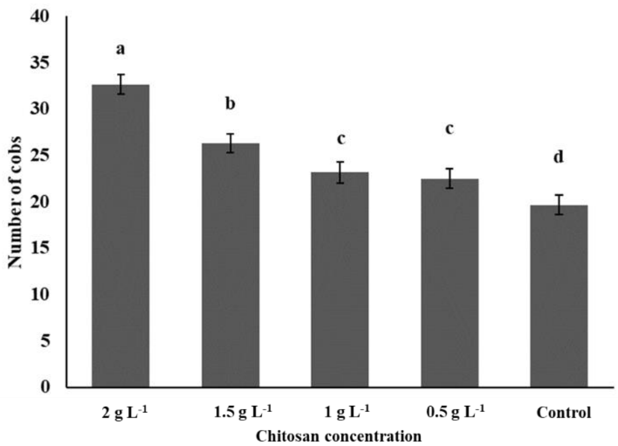

Figure 1. Impact of chitosan on the number of cocoa pods. Different letters in the columns show significant differences according to Tukey's test ($p \leq 0.05$). ± Standard deviation

***Impact of chitosan on the number of almonds*:** The highest concentration of chitosan (2 g L^{-1}), demonstrated the greatest mean number of almonds (averaging 66.5) and exhibited significant differences ($p < 0.05$) compared with the other treatments. A positive correlation was observed between the increase in the chitosan concentration and the number of harvested almonds, indicating the potential beneficial effect of chitosan on cocoa production. In contrast, control treatment presented the lowest yield, with a mean of 52 almonds. All chitosan treatments significantly increased the number of almonds ($p < 0.05$) compared to the control treatment (Figure 2).

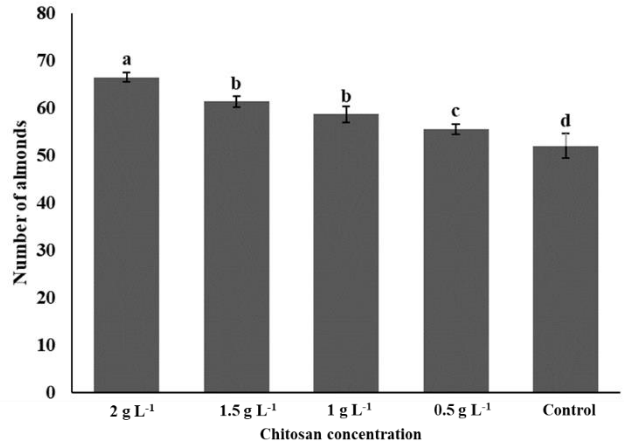

Figure 2. Impact of chitosan on the number of almonds per cocoa pods. Different letters in the columns show significant differences according to Tukey's test ($p \leq 0.05$). ± Standard deviation

***Effects of chitosan on the fresh and dry weight of almonds*:** The treatment with the highest concentration of chitosan, 2 g L^{-1}, demonstrated the greatest fresh and dry weights of almonds, with means of 1936.17 g and 724.7 g, respectively. Regarding fresh weight, the 2 g L^{-1} treatment exhibited statistically significant differences ($p<0.05$) compared to the 1 and 0.5 g L^{-1} treatments, while not showing significant differences ($p>0.05$) with the 1.5 g L^{-1} treatment (Figure 3).

The chitosan treatments with lower concentrations of 1 and 0.5 g L^{-1} yielded the lowest fresh and dry weights, with means of 1790 g and 1780.3 g for fresh weight and 639 g and 619.8 g for dry weight, respectively. In control treatment the average fresh weight and dry weight were 1571 g and 526.2 g, respectively. All chitosan treatments resulted in a significant increase ($p<0.05$) in the fresh and dry weights of harvested almonds compared with the control treatment (Figure 3).

***Effects of chitosan on cocoa yield*:** The application of 2 g L^{-1} chitosan presented the maximum yield (2.45 t ha^{-1}). Chitosan treatment produced a significantly greater yield ($p < 0.05$) than the control group, which had a yield of 0.92 t ha^{-1} (Figure 4). The treatment with the lowest concentration of chitosan, 0.5 g L^{-1}, produced the lowest yield, with a value of 1.59 t ha^{-1}; however, it did not exhibit differences ($p > 0.05$) with the treatments at 1.5 and 1 g L^{-1}. Treatment with 2 g L^{-1} of chitosan was the most effective treatment, demonstrating the highest yield and showing significant differences ($p < 0.05$) compared with the control treatment (Figure 4).

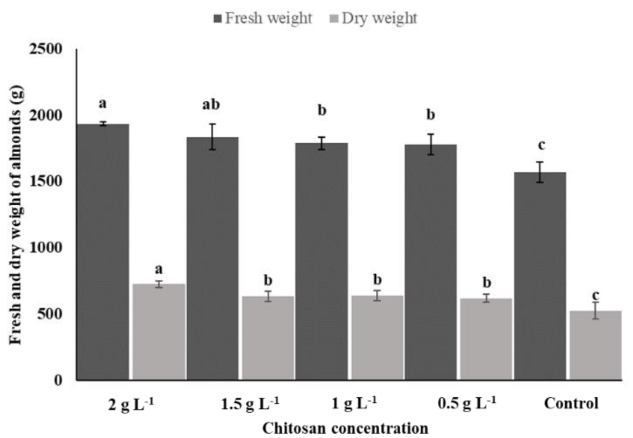

Figure 3. Impact of chitosan on the fresh and dry weight of almonds. Different letters in the columns show significant differences according to Tukey's test ($p \leq 0.05$). ± Standard deviation

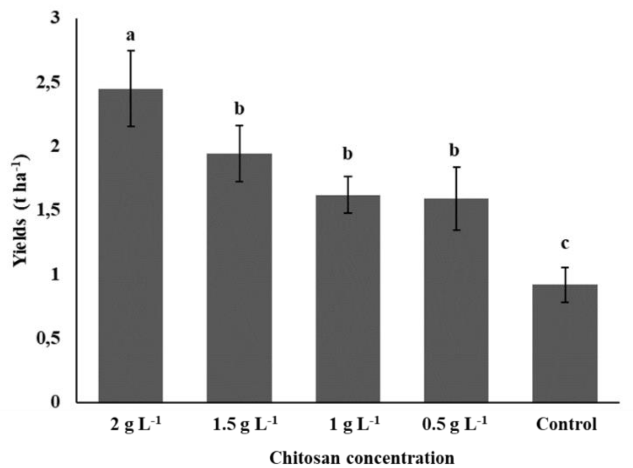

Figure 4. Increase cocoa yield via the application of chitosan. Different letters in the columns show significant differences according to Tukey's test ($p \leq 0.05$). ± Standard deviation

***Incidence of M. roreri infection in cocoa cultivation*:** Regarding the variable incidence of diseases in cocoa crops, ANOVA showed differences ($p < 0.05$) at 60, 120, and 180 days**.** At 60 days, treatment with 2 g L^{-1} exhibited the lowest disease incidence, an average of 11.75%, which was significantly different ($p < 0.05$) from the other treatments. The control group presented the highest disease incidence at 60 days, with an average value of 35.68% (Table 2).

At both 120 and 180 days, the tendency observed at 60 days continued, with the 2 g L^{-1} treatment showing the lowest disease incidences of 14.81 and 21.45%, respectively. The control treatment had the highest disease incidence at both 120 and 180 days, reaching 70.35% by 180 days. All chitosan treatments effectively reduced disease incidence compared with the control treatment (Table 2).

Table 2. Incidence of *M. roreri* disease at 60, 120, and 180 days after chitosan application.

Treatments	Disease Incidence (%)		
	60-DD	120-DD	180-DD
2 g L^{-1}	11.75±1.36a	14.81±1.41a	21.45±0.69a
1.5 g L^{-1}	15.21±0.59b	18.35±1.27b	26.62±1.03b
1 g L^{-1}	18.75±2.52c	22.32±1.65c	30.28±1.50c
0.5 g L^{-1}	21.47±1.38c	25.92±1.34d	34.83±1.36d
Control	35.68±1.93d	42.40±2.13e	70.35±1.32e

Different letters in the columns show significant differences according to Tukey's test ($p \leq 0.05$). ± Standard deviation.

***Severity of M. roreri in cocoa cultivation*:** The variable of disease severity caused by *M. roreri* in cocoa cultivation at 60, 120, and 180 days demonstrated significant differences with respect to chitosan treatment ($p < 0.05$). At all three time points, the 2 g L^{-1} treatment exhibited the lowest severity, which did not show significant differences ($p > 0.05$) compared with the 1.5 g L^{-1} treatment, but did differ significantly from the other treatments. The control treatment exhibited the highest severity on the evaluated days (60, 120, and 180), reaching a severity level of 70.02% (Table 3).

At 180 days, the most effective treatments for reducing disease severity were those at 2 and 1.5 g L^{-1}, which did not differ significantly ($p > 0.05$) from one another. However, the 1.5 g L^{-1} treatment did not significantly differ ($p > 0.05$) from the 1 g L^{-1} treatment. Chitosan treatment at all three time points (60, 120, and 180 days) effectively reduced the severity of *M. roreri* in cocoa cultivation compared with control treatment (Table 3).

Table 3. Severity of *M. roreri* at 60, 120, and 180 days after chitosan application.

Treatments	Disease Severity (%)		
	60-DD	120-DD	180-DD
2 g L^{-1}	6.45±1.00a	11.14±0.94a	18.39±1.06a
1.5 g L^{-1}	8.75±1.04ab	14.30±2.51ab	21.85±2.69ab
1 g L^{-1}	10.66±1.49bc	16.33±3.86bc	24.99±6.04b
0.5 g L^{-1}	13.28±1.98c	19.22±2.10c	34.38±0.99c
Control	±2.38d	39.22±2.48d	70.02±1.32d

Different letters in the columns show significant differences according to Tukey's test ($p \leq 0.05$). ± Standard deviation.

Efficiency of chitosan as a biocontrol: At all three time points, treatment with 2 g L^{-1} concentration (73.65%) did not exhibit differences ($p > 0.05$) to treatment with 1.5 g L^{-1} concentration; however, it was significantly superior ($p < 0.05$) to treatment with 1 and 0.5 g L^{-1} concentrations. At 120 days, the efficiency remained consistent across the various chitosan concentrations, with the 0.5 g L^{-1} being the least effective, maintaining an efficiency of 50%. By 180 days, a

tendency toward increased biocontrol efficiency was observed for all treatments. The 2 g L^{-1} concentration exhibited the highest efficiency (73.73%), whereas the 0.5 g L^{-1} continued to be the least efficient, with an efficiency of 50.99% (Figure 5).

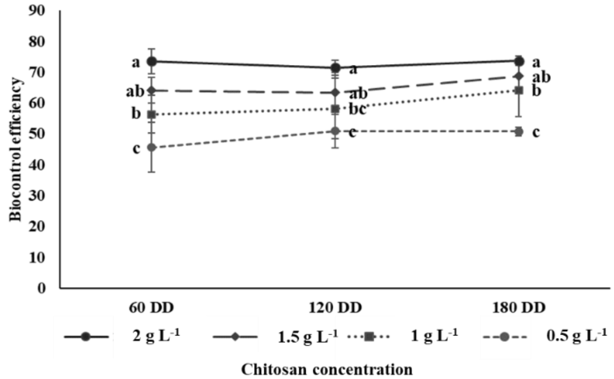

Figure 5. Efficiency of chitosan as a biocontrol agent against *M. roreri* at 60, 120, and 180 days. Different letters on the lines show significant differences according to Tukey's test ($p \leq 0.05$). ± Standard deviation.

The results indicate significant differences in the AUDPC among the treatments ($p < 0.05$). The treatment with 2 g L^{-1} exhibited the lowest AUDPC value of 1,413.60, signifying the highest effectiveness in reducing disease severity. Treatment with 1 g L^{-1} yielded an AUDPC of 2049.40 and did not differ significantly ($p > 0.05$) from 1.5 g L^{-1}, indicating similar efficacy. The 0.5 g L^{-1} treatment led to an AUDPC of 2,582.90, suggesting lower efficacy compared with higher concentrations. The control group, which did not receive chitosan, exhibited the highest AUDPC of 5,187.90, reflecting the greatest disease severity (Figure 6).

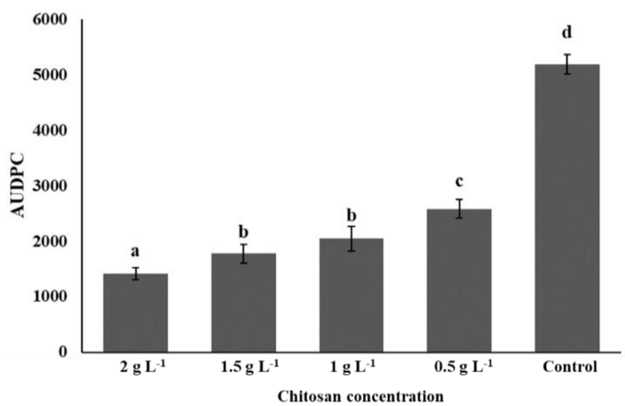

Figure 6. Impact of chitosan on the area under the disease progress curve (AUDPC) of *M. roreri* in cocoa. Different letters in the columns show significant differences according to Tukey's test ($p \leq 0.05$). ± Standard deviation

DISCUSSION

Chitosan as a biostimulant: The best results in the yield indicators in this research were obtained with the highest concentration of chitosan. Pincay-Manzaba *et al.* (2021) reported similar results in tomato cultivation, the authors showed that the highest chitosan concentration increased the crop yield.

Chitosan has been shown to improve yield and its components. As shown by Reyes-Pérez *et al.* (2020), adding chitosan to tomato plants made them make more fruits per plant. Increasing output components is something chitosan can do because it helps plants take in nutrients better and activate their defense mechanisms (Rivas-García *et al*., 2021). This study adding chitosan made the weight of both fresh and dry almonds go up by 123% and 137%, respectively. It's likely that these biostimulant effects are linked to chitosan's ability to help fruit tissue expand which makes the fruit bigger (Chakraborty *et al*., 2020). Similar results to those obtained in this work were reported by Chanaluisa-Saltos *et al.* (2022), the authors found that the application of chitosan increased the fresh and dry weight of tomato fruits.

Chitosan has also been shown to have biostimulant effects in cocoa farming, such as increasing the development of vegetative structures in clones (Reyes-Pérez *et al.*, 2021), reduced germination time, increased germination percentage (Tayo *et al.*, 2017), improved morphophysiological parameters and promoted rooting (Reyes-Pérez *et al*., 2023). Chitosan can enhance the uptake of essential nutrients how well plants, such as nitrogen, phosphorus, and potassium. It can also raise the amount of chlorophyll and optimize the process of photosynthesis (Sharif *et al*., 2018; Shahrajabian *et al.*, 2021). The increase in photosynthetic rate has been reported by Nguyen Van *et al*. (2013). The writers showed that chitosan could raise the amount of chlorophyll in coffee plants and optimize the process of photosynthesis. This rise in photosynthesis makes more energy available for plants to grow and develop (Salachna and Zawadzińska, 2014).

We found that adding chitosan to the cocoa crop increased the yield. The best results in the increase of yields were obtained with the concentration of (2 g L^{-1}). These results are in correspondence with those obtained by the authors Rivas-García *et al*. (2021) and Chanaluisa-Saltos *et al*. (2022) in the tomato crop, the authors also demonstrated that chitosan is an option to increase yields, both authors obtained the highest yields with the highest concentrations of chitosan.

Chitosan as a biocontrol agent against M. roreri in cocoa: All chitosan treatments reduced the incidence and severity of *M. roreri*, demonstrating the potential of chitosan as a biocontrol agent. Incidence and severity were assessed at three-time intervals, at 60, 120 and 180 days, at all times the concentration of (2 gL^{-1}) showed the greatest reduction of the disease.

Chitosan can reduce the incidence and severity of *M. rorei* through different mechanisms of action, such as the direct inhibition of the growth of the phytopathogen. This occurs through the interaction of the amino groups of chitosan with the components of the cell walls of phytopathogens, which causes a rupture of the cell wall and the release of intracellular components, causing cell death (Verlee *et al*., 2017; Tantala *et al*., 2019). Another mechanism is the induction of defense responses in plants and fruits. This biopolymer induces the production of phytoalexins, hypersensitive responses (HR), defense enzymes, the deposition of callose and pathogenesis-related (PR) proteins (de Lamo *et al*., 2021; Torres Rodriguez *et al*., 2021).

These effects were demonstrated in cocoa cultivation by Tayo *et al*. (2017), the authors showed that chitosan reduced the severity and incidence of pod rot caused by the phytopathogen *Phytophthora megakarya*. The application of chitosan in cocoa crops reduced the severity and incidence of pod rot caused by the phytopathogen *Phytophthora megakarya* (Tayo *et al*., 2017), results similar to those obtained in this work, but with the phytopathogen *M. roreri*. In addition, in another research we conducted with chitosan, we found similar results to those of this work, the highest concentration of chitosan (3 g L^{-1}) presented the best results in reducing the incidence and severity of fruit rot caused by *F. oxysporum* (Torres-Rodriguez *et al*., 2024c).

The application of chitosan in coffee crops reduced the severity of rust; the authors demonstrated that plants treated with chitosan presented only 0.2% damage to the leaves, however, the control treatment presented more than 20% damage (Silva-Castro *et al*., 2018). Also, the highest concentration of chitosan (4 g L^{-1}) in potato crops reduced the severity of rot caused by Fusarium species by more than 50% (Mejdoub-Trabelsi *et al*., 2020).

All chitosan treatments in this study reduced the incidence and severity of the disease compared to the control treatment, with the best results obtained with the highest concentration (2 g L^{-1}).

According to the results, this might be because chitosan to inhibit the synthesis of messenger RNA and essential proteins in the cells of phytopathogens (Chopra and Ruhi, 2016; Ke *et al*., 2021). It could also be because chitosan can hold on to metal ions and nutrients such as zinc, copper, cobalt, manganese, nickel, and cadmium, phytopathogenic fungi need them to grow and develop (Kakaei and Shahbazi, 2016; Divya *et al*., 2017).

In this research, the concentration of (2 g L^{-1}) of chitosan was the most efficient in reducing the disease. The area under the disease progression curves (AUDPC) was also the lowest in this case, indicating a slower spread of the disease.

***Conclusions*:** This study demonstrates that chitosan application effectively enhances cacao crop yields and reduces the incidence of frosty pod rot disease. Notably, the 2 g L^{-1} treatment yielded the most favorable outcomes, increasing pod production and significantly lowering disease severity at the 60, 120, and 180-day intervals. These results underline the value of chitosan as a biological alternative to synthetic fungicides, which favours more sustainable and ecologically responsible agricultural practices.

***Authors contributions statement*:** Conceptualization: J.A. Torres-Rodriguez., L.G. Hernandez Montiel and J.J. Reyes-Pérez; methodology: J.A. Torres-Rodriguez, L.G. Hernandez Montiel and J.J. Reyes-Pérez.; validation: P. Preciado Rangel and G. J. Medel-Rodriguez.; writing, J.A. Torres-Rodriguez, K. N. Factos Laiño and G. J. Medel-Rodriguez; review and editing: P. Preciado Rangel; K. N. Factos Laiño and G. J. Medel-Rodriguez; visualization: J.A. Torres-Rodriguez., L.G. Hernandez Montiel; supervision, P. Preciado Rangel; K. N. Factos Laiño and G. J. Medel-Rodriguez; J.A. Torres-Rodriguez and L.G. Hernandez Montiel reviewed and finalized the draft.

***Conflicts of interest*:** The authors declare no conflict of interest.

***Acknowledgments*:** We would like to acknowledge the Universidad Técnica Estatal de Quevedo (UTEQ) and the UTEQ research department for their support.

***Funding*:** The authors did not receive support from any organization for the submitted work. No funding was received to assist with the preparation of this manuscript.

***Ethical statement*:** This article does not contain any studies regarding humans or animals.

***Availability of data and materials*:** All authors affirm that the submitted manuscript is original research which has not been previously published and is not currently under consideration for publication elsewhere.

Informed consent*: *N/A.

***Consent to participate*:** All authors participated in this research study.

***Consent for publication*:** All authors submitted consent to publish this research article in JGIAS.

***SDGs addressed*:** No Poverty, Zero Hunger.

REFERENCES

Abbott, W.S. 1925. A method of computing the ef fectiveness of an insecticide. Journal of Economic Entomology 18:265-267.

Álava, M.A.C., D. Giband and L.C.G. Cruzatty. 2021. Sustainability and Rural Development Through the Production of Organic Cocoa in Calceta – Ecuador: Economic Analysis and Assessment of the Implications

on the Living Standards of Farmers. Proceedings of the 1st International Conference on Water Energy Food and Sustainability pp. 75-82.

Ardean, C., C.M. Davidescu, N.S. Nemeş, A. Negrea, M. Ciopec, N. Duteanu, P. Negrea, D. Duda-Seiman and V. Musta. 2021. Factors Influencing the Antibacterial Activity of Chitosan and Chitosan Modified by Functionalization. International Journal of Molecular Sciences 22:7449.

Avadí, A. 2023. Environmental assessment of the Ecuadorian cocoa value chain with statistics-based LCA. The International Journal of Life Cycle Assessment 28:1495-1515.

Chakraborty, M., M. Hasanuzzaman, M. Rahman, M.A.R. Khan, P. Bhowmik, N.U. Mahmud, M. Tanveer and T. Islam. 2020. Mechanism of Plant Growth Promotion and Disease Suppression by Chitosan Biopolymer. Agriculture 10:624.

Chanaluisa-Saltos, J.S., A.R. Álvarez Sánchez, J.J. Reyes-Pérez and N.A. Lizarde. 2022. Respuesta Agronómica y Fitosanitaria de plantas de Tomate (*Solanum lycopersicum* l.) a la aplicación de Quitosano en condiciones controladas. Revista Científica Agroecosistemas 10:139-145.

Chopra, H. and G. Ruhi. 2016. Eco friendly chitosan: An efficient material for water purification. The Pharma Innovation Journal 5:92-95.

Crespo-Del Campo, E. and F. Crespo-Andia. 1997. Cultivo y beneficio del cacao CCN51. Guayaquil, Paraguay: Editorial El Conejo. ISBN: 9978871756

de Lamo, F.J., M. Šimkovicová, D.H. Fresno, T. de Groot, N. Tintor, M. Rep and F.L. Takken. 2021. Pattern-triggered immunity restricts host colonization by endophytic fusaria, but does not affect endophyte-mediated resistance. Molecular Plant Pathology 22:204-215.

Dhankhar, N. and J. Kumar. 2023. Impact of increasing pesticides and fertilizers on human health: A review. Materials Today: Proceedings 2023.

Divya, K., S. Vijayan, T.K. George and M.S. Jisha. 2017. Antimicrobial properties of chitosan nanoparticles: Mode of action and factors affecting activity. Fibers and Polymers 18:221-230.

Dubin, A., A. Likhanov, O. Klyachenko, A. Subin and A. Kluvadenko. 2020. Effect of chitosan formulations of different biological origin on tobacco (*Nicotiana tabacum* L.) PR-genes expression. Journal of microbiology, biotechnology and food sciences 9:1141-1144.

Espinoza-Lozano, F., D. Amaya-Márquez, C.M. Pinto, M. Villavicencio-Vásquez, D. Sosa del Castillo and S. Pérez-Martínez. 2022. Multiple Introductions of *Moniliophthora roreri* from the Amazon to the Pacific Region in Ecuador and Shared High Azoxystrobin Sensitivity. Agronomy 12:1119.

Faluku, M., T.N.I. Al-Azawi, N.J. Methela, M. Khan, V.N. Huy, A. Brown, D.S. Lee, B.G. Mun, A. Hussian and B.W. Yun. 2024. Fulvic Acid-releasing Chitosan Nanoparticles Promote the Growth and Drought Stress Tolerance of Rice Plants. Journal of Crop Health 76:739-751.

Fuentes, L.F.Q. and S.G. Castelblanco. 2011. Perfil del sabor del clon CCN51 del cacao (*Theobroma cacao* L.) producido en tres fincas del municipio de San Vicente de Chucurí. Publicaciones e Investigación 5:45-58.

Gómez-de la Cruz, I., B. Chávez-Ramírez, C.H. Avendaño-Arrazate, Y.E. Morales-García, J. Muñoz-Rojas and P. Estrada-de los Santos. 2023. Optimization of *Paenibacillus* sp. NMA1017 Application as a Biocontrol Agent for *Phytophthora tropicalis* and *Moniliophthora roreri* in Cacao-Growing Fields in Chiapas, Mexico. Plants 12:2336.

Gong, W., Y. Sun, T. Tu, J. Huang, C. Zhu, J. Zhang, M. Salah, L. Zhao, X. Xia and Y. Wang. 2024. Chitosan inhibits *Penicillium expansum* possibly by binding to DNA and triggering apoptosis. International Journal of Biological Macromolecules 259:129113.

Goudsmit, E., D.M.A. Rozendaal, A. Tosto and M. Slingerland. 2023. Effects of fertilizer application on cacao pod development, pod nutrient content and yield. Scientia Horticulturae 313:111869.

Gustavo González, L., I. Paz, B. Martínez, M. Caridad Jiménez and J.A. Torres Rodriguez. 2015. Respuesta agronómica del cultivo del tomate (*Solanum lycopersicum*, L) var. HA 3019 a la aplicación de quitosana. UTCiencia 2:55-60.

Gutiérrez-Martínez, P., S. Bautista-Baños, G. Berúmen-Varela, A. Ramos-Guerrero and A.M. Hernández-Ibañez. 2017. *In vitro* response of *Colletotrichum* to chitosan. Effect on incidence and quality on tropical fruit. Enzymatic expression in mango. Acta Agronómica 66:282-289.

Hernández, V., M.Á. Botella, P. Hellín, J. Fenoll and P. Flores. 2022. Dose-Dependent Potential of Chitosan to Increase Yield or Bioactive Compound Content in Tomatoes. Horticulturae 8:1152.

Jiménez, D.L., J.C. Alvarez and S. Mosquera. 2022. Frosty pod rot: A major threat to cacao plantations on the move. Tropical Plant Pathology 47:187-200.

Kakaei, S. and Y. Shahbazi. 2016. Effect of chitosan-gelatin film incorporated with ethanolic red grape seed extract and *Ziziphora clinopodioides* essential oil on survival of Listeria monocytogenes and chemical, microbial and sensory properties of minced trout fillet. LWT - Food Science and Technology 72:432-438.

Katiyar, D., A. Hemantaranjan and B. Singh. 2015. Chitosan as a promising natural compound to enhance potential physiological responses in plant: A review. Indian Journal of Plant Physiology 20:1-9.

Ke, C.L., F.S. Deng, C.Y. Chuang and C.H. Lin. 2021. Antimicrobial actions and applications of chitosan. Polymers 13:904.

Lateef, M.A.A., H.K. Hussein and R.A.A.A. Qady. 2023. The application of chitosan in the preservative for fruits and vegetables and control of post-harvest diseases: a review. European Journal of Agricultural and Rural Education 4:20-26.

Lopez-Moya, F., M. Suarez-Fernandez and L.V. Lopez-Llorca. 2019. Molecular Mechanisms of Chitosan Interactions with Fungi and Plants. International Journal of Molecular Sciences 20:332.

Mejdoub-Trabelsi, B., S. Touihri, N. Ammar, A. Riahi and M. Daami-Remadi. 2020. Effect of chitosan for the control of potato diseases caused by *Fusarium* species. Journal of Phytopathology 168:18-27.

Mohan, K., A.R. Ganesan, P.N. Ezhilarasi, K.K. Kondamareddy, D.K. Rajan, P. Sathishkumar, J. Rajarajeswaran and L. Conterno. 2022. Green and eco-friendly approaches for the extraction of chitin and chitosan: A review. Carbohydrate Polymers 287:119349.

Munaro, D., C.H. Mazo, C.M. Bauer, L. da Silva Gomes, E.B. Teodoro, L. Mazzarino, M.B. da Rocha Veleirinho, S.M. e Silva and M. Maraschin. 2024. A novel biostimulant from chitosan nanoparticles and microalgae-based protein hydrolysate: Improving crop performance in tomato. Scientia Horticulturae 323:112491.

Nguyen Van, S., H. Dinh Minh and D. Nguyen Anh. 2013. Study on chitosan nanoparticles on biophysical characteristics and growth of Robusta coffee in green house. Biocatalysis and Agricultural Biotechnology 2:289-294.

Peter, S., N. Lyczko, D. Gopakumar, H.J. Maria, A. Nzihou and S. Thomas. 2021. Chitin and Chitosan Based Composites for Energy and Environmental Applications: A Review. Waste and Biomass Valorization 12:4777-4804.

Pincay-Manzaba, D.F., J.C. Cedeño-Loor and K.A. Espinosa Cunuhay. 2021. Efecto del quitosano sobre el crecimiento y la productividad de *Solanum lycopersicum*. Centro Agrícola 48:25-31.

Rehman, K.U., C. Hollah, K. Wiesotzki, V. Heinz, K. Aganovic, R.U. Rehman, J.I. Petrusan, L. Zheng, J. Zhang, S. Sohail, M.K. Mansoor, C.I. Rumbos, C. Athanassiou and M. Cai. 2023. Insect-derived chitin and chitosan: A still unexploited resource for the edible insect sector. Sustainability 15:4864.

Reyes-Pérez, J.J., E.A. Enríquez-Acosta, M.Á. Ramírez-Arrebato, E. Zúñiga Valenzuela, L. Lara-Capistrán and L.G. Hernández-Montiel. 2020. Efecto del quitosano sobre variables del crecimiento, rendimiento y contenido nutricional del tomate. Revista mexicana de ciencias agrícolas 11:457-465.

Reyes-Pérez, J.J., L.T. Llerena-Ramos, L.G. Hernández-Montiel, V.H. Reynel-Chila, W. Tezara and T. Rivas-García. 2023. Chitosan improves morpho-physiological, rooting attributes and profitability of two cocoa (*Theobroma cacao* L.) varieties during vegetative propagation. Tropical and Subtropical Agroecosystems 26:2023.

Reyes-Pérez, J.J., L.T. Llerena-Ramos, R.A. Ramos-Remache, M.A. Ramírez-Arrebato, A.B. Falcón-Rodríguez, R.A. Pincay-Ganchozo and T. Rivas-García. 2021. Efecto del quitosano en la propagación vegetativa de cacao (*Theobroma cacao* L.) por esquejes. Revista Terra Latinoamericana 39:1-9.

Riseh, R.S., M. Vatankhah, M. Hassanisaadi and J.F. Kennedy. 2023. Chitosan/silica: A hybrid formulation to mitigate phytopathogens. International Journal of Biological Macromolecules 239:124192.

Rivas-García, T., L.G. González-Gómez, T. Boicet-Fabré, M.C. Jiménez-Arteaga, A.B. Falcón-Rodríguez and J.C. Terrero-Soler. 2021. Agronomic response of two tomato varieties (*Solanum lycopersicum* L.) to the application of the biostimulant whit chitosan. Terra Latinoamericana 39:1-9.

Salachna, P. and A. Zawadzińska. 2014. Effect of chitosan on plant growth, flowering and corms yield of potted freesia. Journal of ecological engineering 15:97-102.

Salazar, O.V., S. Latorre, M.Z. Godoy and M.A. Quelal-Vásconez. 2023. The challenges of a sustainable cocoa value chain: A study of traditional and "fine or flavour" cocoa produced by the kichwas in the ecuadorian Amazon region. Journal of Rural Studie 98:92-100.

Salgado-Cruz, M.P., J. Salgado-Cruz, A.B, García-Hernández, G. Calderón-Domínguez, H. Gómez-Viquez, R. Oliver-Espinoza, M.C. Fernández-Martínez and J. Yáñez-Fernández. 2021. Chitosan as a Coating for Biocontrol in Postharvest Products: A Bibliometric Review. Membranes 11:421.

Saravia-Matus, S.L., A.G. Rodríguez and J.A. Saravia. 2020. Determinants of certified organic cocoa production: Evidence from the province of Guayas, Ecuador. Organic Agriculture 10:23-34.

Shahrajabian, M.H., C. Chaski, N. Polyzos, N. Tzortzakis and S.A. Petropoulos. 2021. Sustainable Agriculture Systems in Vegetable Production Using Chitin and Chitosan as Plant Biostimulants. Biomolecules 11:819.

Sharif, R., M. Mujtaba, M.U. Rahman, A. Shalmani, H. Ahmad, T. Anwar, D. Tianchan and X. Wang. 2018. The multifunctional role of chitosan in horticultural crops; a review. Molecules 23:872.

Sikder, M.B.H. and M.M. Islam. 2019. Effect of shrimp chitosan coating on physico-chemical properties and shelf life extension of banana. International journal of engineering technology and sciences 6:41-54.

Silva-Castro, I., R.W. Barreto, M.C.H. Rodriguez, P.M. Matei and J. Martín-Gil. 2018. Control of Coffee Leaf Rust by Chitosan Oligomers and Propolis. "Agriculture for Life, Life for Agriculture" Conference Proceedings 1:311-315.

Sun, W., M.H. Shahrajabian, S.A. Petropoulos and N. Shahrajabian. 2023. Developing Sustainable Agriculture Systems in Medicinal and Aromatic Plant Production by Using Chitosan and Chitin-Based Biostimulants. Plants 12:2469.

Suwanchaikasem, P., S. Nie, A. Idnurm, J. Selby-Pham, R. Walker and B.A. Boughton. 2023. Effects of chitin and chitosan on root growth, biochemical defense response and exudate proteome of *Cannabis sativa*. Plant-Environment Interactions 4:115-133.

Tantala, J., K. Thumanu and C. Rachtanapun. 2019. An assessment of antibacterial mode of action of chitosan on *Listeria innocua* cells using real-time HATR-FTIR spectroscopy. International journal of biological macromolecules 135:386-393.

Tayo, P.M.T., C.A. Ewane, P.O. Effa and T. Boudjeko. 2017. Effects of chitosan and snail shell powder on cocoa (*Theobroma cacao* L.) growth and resistance against black pod disease caused by *Phytophthora megakarya*. African Journal of Plant Science 11:331-340.

Tirado Gallego, P.A., A. Lopera Álvarez and L.A. Ríos Osorio. 2016. Estrategias de control de *Moniliophthora roreri* y *Moniliophthora perniciosa* en *Theobroma cacao* L. Revisión sistemática. Ciencia y Tecnología Agropecuaria 17:417-430.

Torres-Rodriguez, J.A., J.J. Reyes-Pérez, L.H. Adame, B.L. LLerena-Fuentes and L.G. Hernandez-Montiel. 2024a. Marine actinomycetes for biocontrol of *Fusarium solani* in tomato plants: *In vitro* and *in vivo* studies. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 52:13562-13562.

Torres-Rodriguez, J.A., J.J. Reyes-Pérez, M.S. Carranza-Patino, R.R. Gaibor-Fernández, T. Rivas-García and E.O. Rueda-Puente. 2024b. Chitosan: Biocontrol agent of F*usarium oxysporum* in tomato fruit (*Solanum lycopersicum* L.). Emirates Journal of Food and Agriculture 36:1-9.

Torres-Rodriguez, J.A., J.J. Reyes-Pérez, T. Castellanos, C. Angulo, E.E. Quiñones-Aguilar and L.G. Hernandez-Montiel. 2021. A biopolymer with antimicrobial properties and plant resistance inducer against phytopathogens: Chitosan. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 49:12231-12231.

Torres-Rodriguez, J.A., R.A. Ramos-Remache, J. J. Reyes-Pérez, E. F. Quinatoa-Lozada and T. Rivas-García. 2024c. Silicio como bioestimulante en el cultivo de cacao (*Theobroma cacao* L.) y agente de control biológico de *Moniliophthora roreri.* Revista terra latinoamericana 42:1-11.

Verlee, A., S. Mincke and C.V. Stevens. 2017. Recent developments in antibacterial and antifungal chitosan and its derivatives. Carbohydrate polymers 164:268-283.

Verma, K.K., X.P. Song, H.D. Degu, D.J. Guo, A. Joshi, H.R. Huang, L. Xu, M. Singh, D.L. Huang, V.D. Rajput and Y.R. Li. 2023. Recent advances in nitrogen and nano-nitrogen fertilizers for sustainable crop production: a mini-review. Chemical and Biological Technologies in Agriculture 10:111.

Villamil Carvajal, J.E., S.E. Viteri Rosero and W.L. Villegas Orozco. 2015. Aplicación de antagonistas microbianos para el control biológico de *Moniliophthora roreri* Cif & Par en *Theobroma cacao* L. bajo condiciones de campo. Revista Facultad Nacional de Agronomía Medellín 68:7441-7450.

Zhao, J., Q.H. Xue, G.H. Shen, L. Xue, J.L. Duan and D.S. Wang. 2012. Evaluation of *Streptomyces* spp. for biocontrol of gummy stem blight (*Didymella bryoniae*) and growth promotion of *Cucumis melo* L. Biocontrol Science and Technology 22:23-37.